


The role of competition and herbivory in biotic resistance against invaders: a synergistic effect

JORGE SANTAMARÍA ¹, FIONA TOMAS,² ENRIC BALLESTEROS,³ JUAN M. RUIZ,⁴ JAIME BERNARDEAU-ESTELLER,⁴
JORGE TERRADOS,² AND EMMA CEBRIAN^{3,5}

¹Marine Resources and Biodiversity Research Group (GRMAR), Institute of Aquatic Ecology, University of Girona, Girona, 17003 Catalonia, Spain

²Marine Ecosystems Dynamics Group—Instituto Mediterráneo de Estudios Avanzados (IMEDEA), Universitat de les Illes Balears (UIB)—Consejo Superior de Investigaciones Científicas (CSIC), Esporles, 07190 Balearic Islands, Spain

³Centre d'Estudis Avançats de Blanes, Consejo Superior de Investigaciones Científicas (CEAB-CSIC), Blanes, 17300 Catalonia, Spain

⁴Seagrass Ecology Group—Centro Oceanográfico de Murcia, Instituto Español de Oceanografía (IEO), San Pedro del Pinatar, 30740 Murcia, Spain

Citation: Santamaría, J., F. Tomas, E. Ballesteros, J. M. Ruiz, J. Bernardeau-Esteller, J. Terrados, and E. Cebrian. 2021. The role of competition and herbivory in biotic resistance against invaders: a synergistic effect. Ecology 00(00):e03440. 10.1002/ecy.3440

Abstract. Invasive species pose a major threat to global diversity, and once they are well established their eradication typically becomes unfeasible. However, certain natural mechanisms can increase the resistance of native communities to invaders and can be used to guide effective management policies. Both competition and herbivory have been identified as potential biotic resistance mechanisms that can limit plant invasiveness, but it is still under debate to what extent they might be effective against well-established invaders. Surprisingly, whereas biotic mechanisms are known to interact strongly, most studies to date have examined single biotic mechanisms separately, which likely influences our understanding of the strength and effectiveness of biotic resistance against invaders. Here we use long-term field data, benthic assemblage sampling, and exclusion experiments to assess the effect of native assemblage complexity and herbivory on the invasion dynamics of a successful invasive species, the alga *Caulerpa cylindracea*. A higher complexity of the native algal assemblage limited *C. cylindracea* invasion, probably through competition by canopy-forming and erect algae. Additionally, high herbivory pressure by the fish *Sarpa salpa* reduced *C. cylindracea* abundance by more than four times. However, long-term data of the invasion reflects that biotic resistance strength can vary across the invasion process and it is only where high assemblage complexity is concomitant with high herbivory pressure, that the most significant limitation is observed (synergistic effect). Overall, the findings reported in this study highlight that neglecting the interactions between biotic mechanisms during invasive processes and restricting the studied time scales may lead to underestimations of the true capacity of native assemblages to develop resistance to invaders.

Key words: alga–herbivore interactions; biological invasions; biotic resistance; *Caulerpa cylindracea*; herbivory; interspecific competition; invasion ecology; *Sarpa salpa*.

INTRODUCTION

Biological invasions are one of the main threats to biodiversity and ecosystem function worldwide, being the second most prominent cause of species extinctions and playing an important role in diversity reduction (Vilà et al. 2011, Bellard et al. 2016). Furthermore, bioinvasions can produce alterations in a number of ecosystem services and basic ecosystems processes (Pejchar and Mooney 2009, Vilà et al. 2010, Simberloff et al. 2013), often at great economic cost (Pimentel et al. 2005). Still, our understanding of the factors that

influence invasion success remains limited (Simberloff et al. 2013), complicating the development of effective management strategies to prevent and mitigate the negative effects of invasive species.

The success of an invasion is dependent on multiple processes across a wide range of temporal and spatial scales (Perelman et al. 2007, Theoharides and Dukes 2007, Eschtruth and Battles 2009a, Byun et al. 2015). Among these processes, most of the attention has fallen on biological processes, in the context of the Biotic Resistance Hypothesis (Elton 1958, Keane and Crawley 2002, Levine et al. 2004). The strength of biotic resistance against an invader is strongly influenced by the native assemblage and by the functional traits of the native species (Pokorny et al. 2005, Perelman et al. 2007, Byun et al. 2013), which modulate the interspecific

Manuscript received 17 March 2021; accepted 5 April 2021.
Corresponding Editor: Michael H. Graham.

⁵Corresponding Author. E-mail: emma@ceab.csic.es

competition; but also by the consumer pressure on both the invasive and the native species (Levine et al. 2004, Parker and Hay 2005, Mitchell et al. 2006). In this sense, negative effects of competition on several invasive plants, mainly caused by the limitation of essential resources such as water, nutrients or light, have been previously reported in tropical forests (Fine 2002), salt-marshes (Amsberry et al. 2000), grasslands (Corbin and D'Antonio 2004, te Beest et al. 2018), mangroves (Li et al. 2014, Zhang et al. 2018), shrublands (Morris et al. 2015), and freshwater ecosystems (Petrizzella et al. 2018). However, competition alone might not be enough to exert a strong biotic control against a well-established invader (Levine et al. 2004, Vilà and Weiner 2004). On the other hand, herbivory has been also acknowledged as an important biotic resistance mechanism for native ecosystems (Levine et al. 2004, Parker and Hay 2005, Parker et al. 2006), although its effectiveness is controversial (Maron and Vilà 2001, Keane and Crawley 2002, Liu and Stiling 2006). In fact, herbivores can promote (Eschtruth and Battles 2009b, Relva et al. 2010, Kalisz et al. 2014), deter (Cushman et al. 2011, Pearson et al. 2012, Zhang et al. 2018), or have no effect on the dominance of invasive plant species (Stohlgren et al. 1999), which makes it difficult to understand the conditions under which herbivory can be an effective biotic resistance mechanism against an invasion. Taking into account that herbivory can be highly influenced by other factors such as native plant traits (Grutters et al. 2017) or habitat features (Alofs and Jackson 2014, Li et al. 2014, Ender et al. 2017, Zhang et al. 2018), contrasting observations on the role of biotic mechanisms in controlling invasive species may be partially explained by the fact that they are often assessed neglecting the relative importance of the interactions between mechanisms (Levine et al. 2004, Mitchell et al. 2006, Alofs and Jackson 2014, Zhang et al. 2018, Petrizzella et al. 2020). Indeed, the interaction between biotic mechanisms has been suggested to be responsible for an enhancement in the biotic resistance capacity of the invaded community (Suwa and Louda 2012, Li et al. 2014, Zhang et al. 2018).

In marine ecosystems, macroalgae are one of the most conspicuous and successful invaders, as well as one of the most harmful, comprising 20% of marine invasive species worldwide (Schaffelke et al. 2006) and causing potentially important ecological and economic damage (Williams and Smith 2007). Despite this, the factors that drive their invasive success remain largely unknown (Inderjit et al. 2006), although similarly to what has been observed for plant invasions in terrestrial ecosystems, both competition and herbivory are suspected to determine their invasion success (Kimbrow et al. 2013, Papacostas et al. 2017). In this regard, functional traits of the native species can influence habitat resistance to algae invasion through their contribution to interspecific competition (Arenas et al. 2006, Britton-Simmons 2006, Vaz-Pinto et al. 2012).

Surprisingly, however, most studies seem to confirm the inability of herbivores to limit the spread of well-established invasive macroalgae (e.g., Wikström et al. 2006, Forslund et al. 2010, Cebrian et al. 2011, Tomas et al. 2011b, Nejrup et al. 2012), despite the important role of herbivory regulating algal abundance and distribution (Vergés et al. 2009, Poore et al. 2012). Overall, previous research on biotic resistance against invasive macroalgae seems to suggest that, in most cases, the effect of single biotic mechanisms might not be enough to significantly affect invader performance (Kimbrow et al. 2013, Papacostas et al. 2017). Probably, as has been suggested above, only by considering the interaction between biotic mechanisms (both competition and herbivory), can more robust conclusions on the true resistance of an assemblage towards a particular invader be obtained.

In this study, we aim to test whether herbivory interacts with competition to modulate the resistance of a marine habitat towards a particular invader. We use *Caulerpa cylindracea*, one of the most invasive macroalgae worldwide, to assess the simultaneous role that both assemblage structure and herbivory pressure have on *C. cylindracea* invasion by using in situ field experiments that assess herbivory and assemblage characteristics. Additionally, we monitor the abundance of the invader over time to further elucidate whether the studied biotic mechanisms and their interaction influence the long-term dynamics of the invader.

MATERIALS AND METHODS

Study species

Caulerpa cylindracea is a green alga, native to the Southwestern coast of Australia (Verlaque et al. 2003), which is currently considered one of the most invasive species within the Mediterranean Sea (Klein and Verlaque 2008, Katsanevakis et al. 2016), having also invaded areas in the North Atlantic (Verlaque et al. 2004). Actually, it has recently been ranked as one of the marine invaders with the highest negative ecological impacts worldwide (Anton et al. 2019). However, despite its formidable ability to spread and grow, the abundance of *C. cylindracea* appears to differ markedly among invaded assemblages (Klein and Verlaque 2008, Cebrian and Ballesteros 2009), suggesting that there might be, in some cases, some natural mechanisms controlling *C. cylindracea* abundance. Among such mechanisms, competition may play an important role, because canopy-forming and erect algae (typical of high-complexity assemblages) can outcompete *C. cylindracea* (Ceccherelli et al. 2002, Bulleri and Benedetti-Cecchi 2008, Piazzini and Balata 2009, Bulleri et al. 2010), whereas herbivory seems to fail as a control mechanism for *C. cylindracea* when it is well established (Bulleri et al. 2009, Cebrian et al. 2011), even though several species are known to feed on it commonly (Ruitton et al. 2006, Box et al. 2009,

Cebrian et al. 2011, Tomas et al. 2011b). However, it is important to consider that previous studies have focused on only one of these mechanisms (either competition or herbivory) while, in nature, both mechanisms might act together to influence the abundance of the invasive species.

Study system

The Mediterranean Sea is the largest and deepest semienclosed sea on Earth and it is considered a hotspot for marine biodiversity as it harbors around 17,000 marine species, 20% of them being endemic to the region (Coll et al. 2010). Because of its temperate climatic conditions, Mediterranean benthic shallow habitats are dominated by macroalgae. Unfortunately, the Mediterranean Sea is one of the areas most susceptible to the introduction of nonnative species worldwide (Galil 2007) and it is considered to be a hotspot for invasive algae (Williams and Smith 2007, Thomsen et al. 2016).

The study was conducted in the Cabrera Archipelago National Park, in the Balearic Islands (western Mediterranean; 39°12'21" N, 2°58'44" E; Fig. 1). This maritime-terrestrial national park was established in 1991 and currently harbors some of the best-preserved benthic and fish assemblages in the Mediterranean Sea (Sala et al. 2012, Guidetti et al. 2014).

The invasive alga, *C. cylindracea*, was first detected in the area in 2003 at a depth of 30 m (Cebrian and Ballesteros 2009) and has, since then, colonized most of the park's benthic habitats at depths from 0 to 65 m. Indeed, in some of these habitats, it has become the dominant species (Cebrian et al. 2011).

Benthic habitat sampling and assemblage complexity

To assess the role that benthic assemblage complexity might have on *C. cylindracea* coverage, different assemblages were surveyed in three sites around the Cabrera Archipelago: Ses Rates, Na Foradada, and Freu de la Imperial (Fig. 1). A shallow assemblage (10 m) and a deep assemblage (30 m) were surveyed at each site to take into account the wide range of benthic assemblage complexities (Ballesteros et al. 1993) and contrasting herbivory pressures (Vergés et al. 2009, Tomas et al. 2011b) in relation to depth. Assemblages were sampled in 2005, 2006, and 2007. At each site and depth, three random samples measuring 20 × 20 cm² were collected, with the whole benthic cover removed using a hammer and a chisel (Boudouresque 1971, Sant et al. 2017). After removing the erect algae, and before scraping each quadrat, the cover of each encrusting species was visually estimated to obtain a more reliable value of their abundance in the encrusting layer. After collection, samples were preserved in 4% formalin in seawater, and once in the laboratory, they were sorted and all algae were identified to species level. Species coverage was calculated by placing the species specimens horizontally over a laboratory

tray and measuring the area they covered (Ballesteros 1986). Then, each algal species was assigned to a different category ("Canopy-forming," "Erect," "Turf," and "Encrusting") based on their morphological traits (size and morphology; Appendix S1: Table S1). In order to avoid the effect of assemblage complexity being subject to a confounding effect of the presence of *C. cylindracea*, this species was not included in any of the previous categories. Finally, the percent cover of each category in the sample was calculated.

Capacity of canopy-forming and erect algae to out-compete *C. cylindracea* in contrast to turf and encrusting species has been experimentally proven for coastal shallow rocky bottoms of the Mediterranean Sea (Ceccherelli et al. 2002, Bulleri and Benedetti-Cecchi 2008, Piazzini and Balata 2009, Bulleri et al. 2010). Consequently, complexity of each sample was defined based on the percentage abundance of the "Canopy-forming" and "Erect" categories. Three levels of complexity were defined for the samples based on the percentage of coverage that comprised canopy-forming and/or erect species: "high complexity" (more than 50%); "medium complexity" (between 15 and 50%), and "low complexity" (lower than 15%).

Exclusion experiment

An exclusion experiment was performed to assess whether fish herbivory could act as a biotic resistance mechanism against *C. cylindracea* invasion by reducing the abundance of the invasive alga. To this end, in order to obtain a proxy of contrasting herbivory intensities, and bearing in mind that herbivory pressure decreases strongly through the water column (Vergés et al. 2009, 2012, Steneck et al. 2017), the exclusion experiment was performed at two different depths: 10 m, where herbivory pressure is high, and 30 m, where it is low (Reñones et al. 1997, Tomas et al. 2011b, Vergés et al. 2012).

This experiment mainly targeted *Sarpa salpa*, because it is the only truly herbivorous fish in the western Mediterranean Sea (Verlaque 1990, Gianni et al. 2017), it plays an important role structuring algal communities (Vergés et al. 2009) and it regularly consumes *C. cylindracea* (Ruitton et al. 2006, Tomas et al. 2011b). This fish species is quite abundant throughout the Cabrera Archipelago, where it can reach densities up to 14 individuals per 250 m² above depths of 20 m (Reñones et al. 1997), spending around 90% of the time above 20 m (Tomas et al. 2011b). *Sarpa salpa* is known to have a quite heterogeneous diet (Havelange et al. 1997), but it can also be very selective (Verlaque 1990, Marco-Méndez et al. 2017) and even shows a preference for *C. cylindracea* over many native species (Tomas et al. 2011b), which makes it a potential candidate for the control of *C. cylindracea*.

The exclusion experiment was set up at the end of June 2011 in Na Foradada (Fig. 1), an area where fish

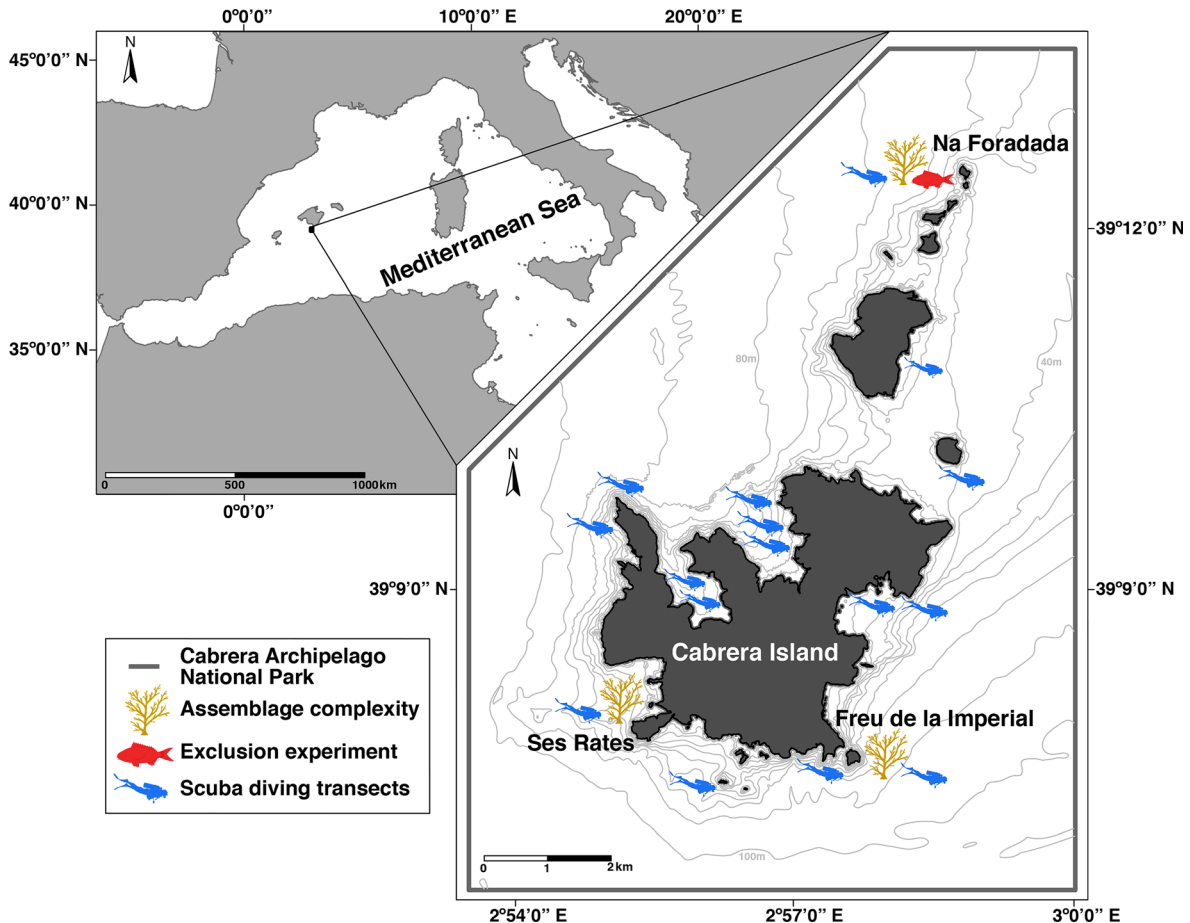


FIG. 1. Location of the Cabrera Archipelago National Park. The points show the locations of the sampling sites where assemblage complexity was assessed (3 sites), the exclusion experiment was performed (1 site) and the scuba diving transects were done (16 sites). Shapefiles for the Mediterranean Sea www.naturalearthdata.com and for the Cabrera Archipelago National Park www.miteco.gob.es.

communities are well established, sea urchin (*Paracentrotus lividus* and *Arbacia lixula*) densities are very low ($<0.1/\text{m}^2$) (Cebrian et al. 2011) and the highest densities of the fish *S. salpa* are found within the National Park, with more than 20 individuals per 250 m^2 (Reñones et al. 1997). Furthermore, we chose this area because both the shallow and the deep benthic habitats displayed a similar medium complexity (with coverage of erect and canopy-forming species at between 25% and 35%), and similar abundances of native species. This meant there was sufficient abundance of *C. cylindracea* to assess, in the field, the effect of herbivory pressure on it.

At each depth, three treatments were used: “Exclusion,” which consisted of cages of $50 \times 50 \times 50\text{ cm}^3$ made of plastic netting with a mesh size of 2.5 cm; “Control-Exclusion,” consisting of cages with open sides; and “Control,” consisting of $50 \times 50\text{ cm}^2$ quadrats marked permanently on the corners and without a cage. A total of five interspersed replicates per treatment were set (15 plots per depth) within an area of $<100\text{ m}^2$ to

avoid different abiotic conditions between plots. At the beginning (July) and at the end (August) of the experiment, pictures were taken at each plot to assess *C. cylindracea* abundance subsequently, which was calculated with the computer program *photoQuad* version 1.4 (Trygonis and Sini 2012). In each photograph, 50 random points were placed and then, each of these points was assigned to the category of either “*C. cylindracea*” or “other algae.” The proportion of points in each category was then used as a proxy of the percentage abundance for each of those two categories.

Abundance of *Caulerpa cylindracea*

The abundance of *C. cylindracea* at the Cabrera Archipelago was assessed in 2008 and then again in 2017. To do so, 16 representative sites around the archipelago were chosen (Fig. 1) and a perpendicular transect to shore was performed at each site by means of scuba diving. The depth of the transects ranged from 5 to 45 m to

cover the main bathymetric range at which *C. cylindracea* can be found (Cebrian et al. 2011). The abundance of *C. cylindracea* was estimated by means of $25 \times 25 \text{ cm}^2$ quadrats, divided into 25 subquadrats of $5 \times 5 \text{ cm}^2$ (Sala and Ballesteros 1997, Sant et al. 2017) and the number of subquadrats where *Caulerpa* was detected, was used as a unit of abundance. A total of 30 quadrats were randomly positioned within each 5-m-depth range, and then the mean *C. cylindracea* abundance per each 5-m-depth stratum was calculated. Also, to take into account the effect that assemblage complexity might have on the bathymetric abundance of *C. cylindracea*, each 5-m-depth range at each site was classified as having either high or low complexity based on the dominant morphological categories in the assemblage (“canopy-forming,” “erect,” “turf,” and “encrusting”), while ignoring the presence of *C. cylindracea* to avoid confounding effects.

Statistical analysis

The effect of benthic assemblage complexity on the cover of *C. cylindracea* was assessed with binomial generalized linear models (GLM), because the response variable was measured as a percentage (percentage of *C. cylindracea* coverage in each sample) and it could be approximated to a logistic distribution (e.g., success = % coverage of *C. cylindracea*, failure = % coverage of species other than *C. cylindracea*). Two models were fitted, one to assess the role of “assemblage complexity” and another to assess the role of both “depth” and “assemblage complexity” on *C. cylindracea* coverage. In the latter, both factors were included as fixed effects, and if the interaction between them was significant, it was also included in the model. To fit both models, the statistical environment R was used (R version 3.6.3) (R Development Core Team 2018); and to compare the effects between levels in the assemblage complexity factor itself (“high complexity,” “medium complexity,” and “low complexity”), and in the assemblage complexity factor at each depth (“shallow” and “deep”), Tukey post hoc tests were performed using the functions “pairs” and “emmeans” from the package *emmeans* (Lenth 2018).

To evaluate the effect of the exclusion treatment on the abundance of *C. cylindracea* at the end of the experiment, binomial mixed-effects GLMs were used because the response variables were measured as proportions and could be approximated to a logistic distribution (e.g., success = points that corresponded to *C. cylindracea*; failure = points that did not correspond to *C. cylindracea*), and the random terms were used to take into account the repeated measures. In the models, the factors “treatment” and “time” were included as fixed effects, whereas “plot” was included as a random effect. If the interaction between “treatment” and “time” was significant, it was also included in the fixed part of the model. Two models were fitted, one per depth (10 or 30 m) by means of the package *lme4* (Bates et al. 2015) for

R. Tukey post hoc tests, which were performed using the functions “pairs” and “emmeans” in the package *emmeans*, were used to compare effects between levels in the treatment factor (“Exclusion,” “Control-Exclusion,” “Control”) at each time observation (“beginning” and “end”).

The R code used to perform all the statistical analyses can be found on Zenodo⁶ (Santamaria 2021).

RESULTS

Effect of benthic assemblage complexity on *Caulerpa cylindracea* coverage

Benthic assemblage complexity had a significant effect on *C. cylindracea* coverage ($P < 0.001$, Appendix S2: Table S1), with high complexity assemblages supporting low *C. cylindracea* covers (Fig. 2A). In fact, there were significant differences in *C. cylindracea* cover among the different levels of assemblage complexity ($P < 0.001$, Appendix S2: Table S2), with *C. cylindracea* coverage lower than 5% in very complex assemblages, whereas in low-complexity assemblages, coverage of the invasive species reached values of almost 30% (Fig. 2A).

When the depth of the sampled assemblages was included in the model, *C. cylindracea* coverage varied significantly by depth, by assemblage complexity and by the interaction term (Appendix S2: Table S3). In fact, although the previous pattern of lower *C. cylindracea* cover in high complexity assemblages is maintained, the coverage of the invasive alga is significantly higher at deeper habitats, independently of assemblage complexity (Appendix S2: Table S4, upper table; Fig. 2B). In particular, in shallow habitats, high-complexity assemblages had 20 times less *C. cylindracea* coverage than low complexity assemblages, but only 10 times less coverage at deeper habitats (Fig. 2B). At each depth, there were significant differences between all levels of assemblage complexity, except between high complexity and medium complexity assemblages in shallow areas (Appendix S2: Table S4, lower table).

Exclusion experiment

Significant effects of herbivory on *C. cylindracea* abundance were only detected at shallow depths (10 m), where *C. cylindracea* abundance varied significantly by treatment, by time and by the interaction between the two (Appendix S3: Table S1). Although at the beginning of the experiment, all treatment areas displayed similar *C. cylindracea* abundance, both the “Control-Exclusion” and the “Control” treatments exhibited lower *C. cylindracea* abundances at the end of the experiment ($P < 0.0001$; Appendix S3: Table S2, upper table), whereas *C. cylindracea* abundance in the “Exclusion” treatment remained constant (Appendix S3: Table S2, lower table).

⁶ <https://doi.org/10.5281/zenodo.4664432>

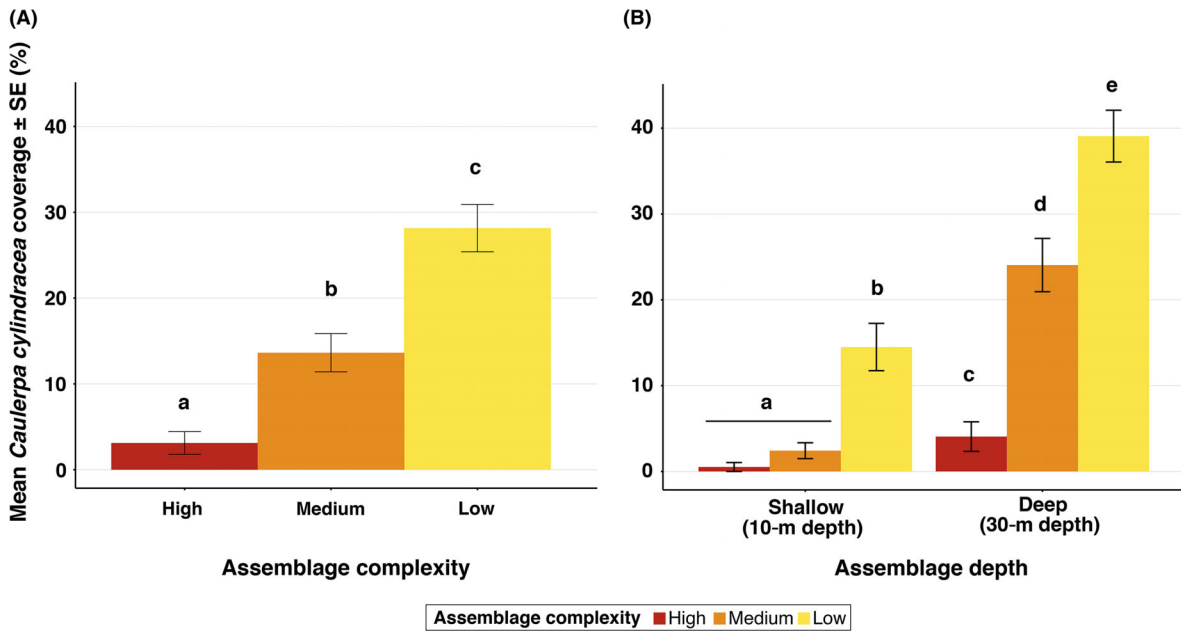


FIG. 2. Mean *Caulerpa cylindracea* coverage \pm SE (A) for each level of assemblage complexity, and (B) for each level of assemblage complexity at the two different depths. Significant differences between assemblage complexity levels (P values from Tukey's test with 95% confidence intervals) are indicated with letters.

Indeed, at the end of the experiment, *C. cylindracea* abundance was 4.33 and 2.36 times higher in the full exclusion cages compared with the uncaged control plots and the side-open cages, respectively (Fig. 3A).

On the other hand, at 30 m, the abundance of *C. cylindracea* varied significantly across time but not between treatments (Appendix S3: Table S1). In fact, for all three treatment levels, abundance was significantly higher (1.88 times on average) at the end of the experiment, than at the beginning (Fig. 3B).

Abundance of *Caulerpa cylindracea*

The abundance of *C. cylindracea* in the Cabrera Archipelago decreased between 2008 and 2017 at depths of between 5 and 35 m, but remained more or less constant below 40 m (Fig. 4). In the entire bathymetric distribution, assemblage complexity showed a considerable effect on *C. cylindracea* abundance, with high-complexity assemblages exhibiting lower abundances of the invasive alga than low complexity assemblages. Furthermore, it was in shallow and highly complex assemblages, from 5 to 25 m deep, where *C. cylindracea* reached its lowest abundance, with values ranging between 5 and 10% (Fig. 4B).

DISCUSSION

Our results indicate that although both competition and herbivory can provide biotic resistance to a certain extent, strong synergistic effects are observed when the

two mechanisms act together. Indeed, competition (by canopy-forming and erect algae) and herbivory (by *Sarpa salpa*), significantly affect the invasion outcomes of *C. cylindracea*, particularly limiting its abundance in complex algal assemblages in which herbivory pressure is high.

Benthic assemblage complexity had a strong influence on preventing *C. cylindracea* invasion. The dominance of canopy-forming and erect species resulted in reduced cover of *C. cylindracea*, probably determining resistance to *C. cylindracea* invasion through competition mechanisms (Ceccherelli et al. 2002, Piazzini and Balata 2009), such as the reduction in light availability, which can limit the photosynthetic performance of *C. cylindracea* (Bernardeau-Esteller et al. 2015, 2020, Marín-Guirao et al. 2015) and the prevention of reattachment of fragments through the branch-sweeping of the substratum (Bulleri and Benedetti-Cecchi 2008, Piazzini et al. 2016). In contrast, when the abundance of canopy-forming algae was low and the communities were dominated by turf and encrusting species, *C. cylindracea* cover was much higher. In such habitats, the colonization and spread of *C. cylindracea* seems to be facilitated because turf assemblages provide an optimal 3-D matrix that favors the anchoring of the stolons and trapping of fragments (Ceccherelli et al. 2002, Bulleri and Benedetti-Cecchi 2008, Bulleri et al. 2009). In this sense, these findings support previous evidence from marine and terrestrial ecosystems, where the presence and dominance of species with certain functional traits (e.g. growth form, size and height) among the assemblage, strongly

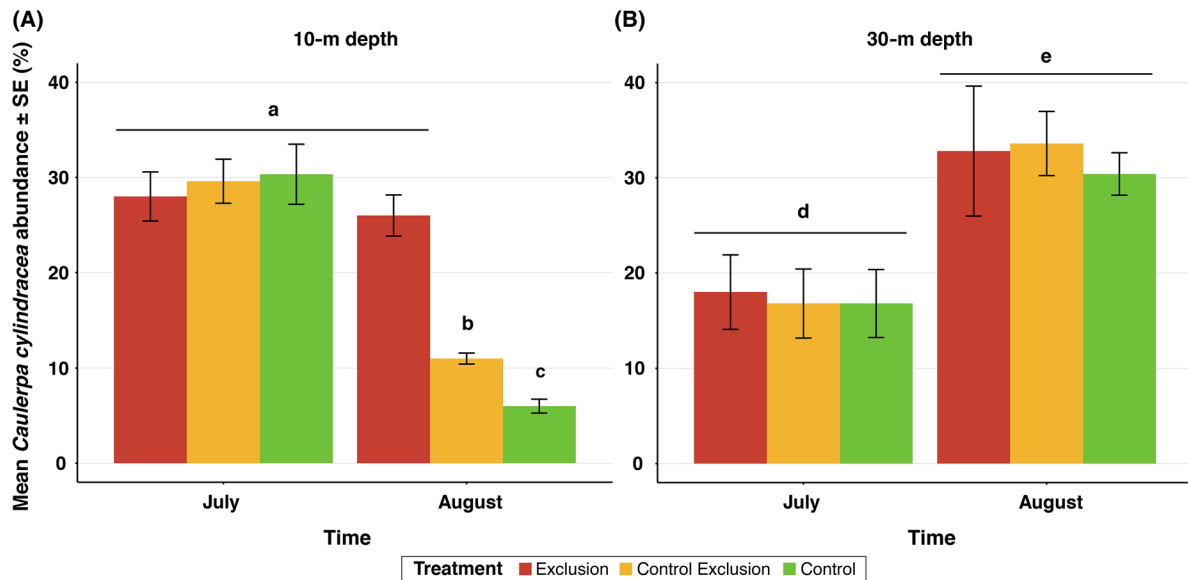


FIG. 3. Mean *Caulerpa cylindracea* abundance \pm SE, at each time observation and for each treatment in the exclusion experiment. (A) Exclusion experiment at 10-m depth and (B) exclusion experiment at 30-m depth. Significant differences between exclusion treatments (P -values from Tukey's test with 95% confidence intervals) are indicated with letters in each graph.

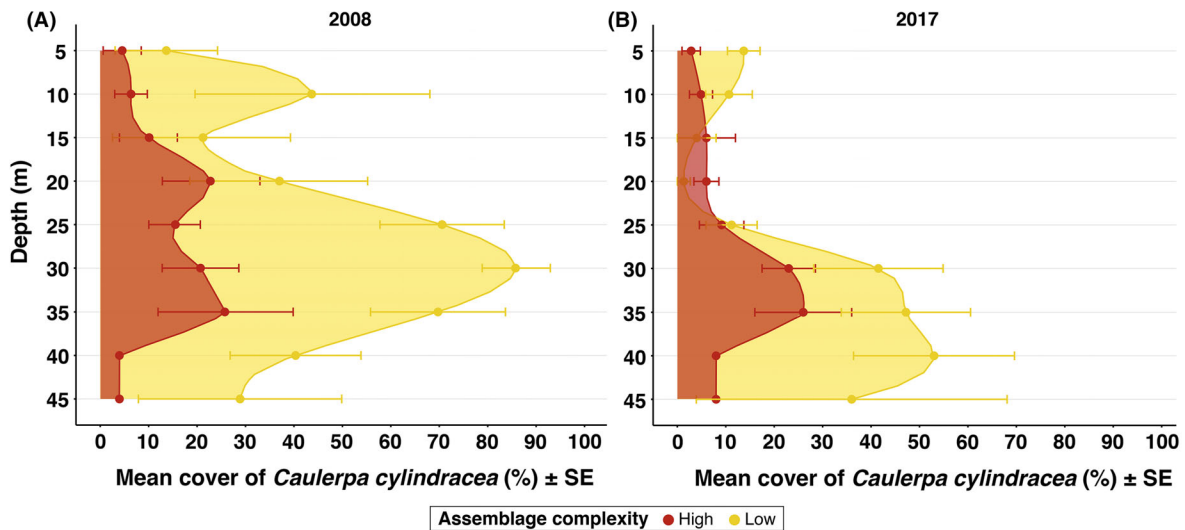


FIG. 4. Bathymetric abundance of *Caulerpa cylindracea* (mean \pm SE) at the Cabrera Archipelago National Park: (A) in 2008 and (B) in 2017, on assemblages with different complexities.

influences the strength of the biotic resistance against invasive primary producers (Lindig-Cisneros and Zedler 2002, Arenas et al. 2006, Britton-Simmons 2006, Byun et al. 2013, Bernardeau-Esteller et al. 2020) by limiting one or several essential resources for the invader.

However, the significant differences we observed in the abundance of *C. cylindracea* between shallow and deep communities, regardless of assemblage complexity, suggest that mechanisms other than assemblage

complexity are playing an important role on the invasion of *C. cylindracea*, particularly given that *C. cylindracea* can readily colonize habitats from 0 to 50 m depth (Klein and Verlaque 2008, Cebrian and Ballesteros 2009). In this sense, although several factors (e.g., propagule pressure, disturbance, or abiotic conditions) cannot be disregarded, we suggest that the observed differences in invader abundance mainly reflect the contrasting consumer pressures found between deep and shallow

communities. Concretely, given similar assemblage complexity, when herbivory pressure was high (here shallow habitats) (Reñones et al. 1997, Vergés et al. 2009, 2012, Tomas et al. 2011b), the abundance of *C. cylindracea* was significantly lower compared to areas where herbivory pressure was low or nonexistent (deeper habitats). Actually, both the exclusion experiment and the pattern of *C. cylindracea* abundance and distribution across the Cabrera Archipelago further support that herbivory is also contributing to the biotic resistance of native assemblages to the *C. cylindracea* invasion. In this sense, where herbivory is weak, such as in deep habitats, or if herbivores have been depleted, *C. cylindracea* is subject to limited control and its abundance depends largely on assemblage complexity, which results in higher abundances of the invader among the native assemblage (Fig. A, B). Similarly, when herbivory pressure is high but assemblage complexity is low, the invader will also suffer limited control (Fig. 5C). However, when high herbivory pressure is concomitant with high assemblage complexity, both mechanisms (competition and herbivory) strongly limit the abundance of *C. cylindracea* (Fig. 5D). Taking into account that sea urchin densities were very low in the study area (<0.1 per m^2), the herbivory pressure observed can be mainly attributed to the effect of *Sarpa salpa*, a fish that is mostly distributed at shallow depths and has a certain preference for *C. cylindracea* (Tomas et al. 2011b). Nevertheless, considering that sea urchins have been previously reported feeding on *C. cylindracea* (Ruitton et al. 2006, Bulleri et al. 2009, Cebrian et al. 2011, Tomas et al. 2011a), additional negative effects on *C. cylindracea* abundance may occur in areas with high sea urchin abundances. These results where the interaction between biotic mechanisms yielded a stronger biotic resistance against the invader than the single mechanisms alone, agree with previous studies where habitat characteristics interacted with herbivory pressure to influence the overall biotic resistance of certain terrestrial ecosystems (Suwa and Louda 2012, Li et al. 2014, Zhang et al. 2018).

Also, and importantly, by following the long-term dynamics of *C. cylindracea* at the study area, we observe that the overall strength of the assemblage's biotic resistance has increased over time as the abundance of the invasive alga in assemblages subjected to higher biotic resistance (i.e., communities at depths of between 0 and 25 m), has decreased over a 10-yr period (Fig. 4). This regression, restricted to areas with high herbivore pressure, may be the result of either an increase in the abundance of the herbivores or to some herbivores becoming more efficient in consuming the invasive species as the invasion progresses (Strayer et al. 2006, Carlsson et al. 2009). However, given that the abundance of *S. salpa* has remained more or less stable during the assessed period (Coll 2020), we suspect that this herbivore has become more efficient at targeting the invader and has increased its per capita consumption rates over time (J. Santamaría, *unpublished manuscript*). Nevertheless, other

mechanisms that can increase biotic resistance, such as, for instance, allelochemical defenses deployed by native species and assemblages against the invader (Strayer et al. 2006), cannot be ruled out.

Unfortunately, in general, knowledge on whether the effects of biotic mechanisms are maintained throughout an invasion process or whether they change in relation to time since invasion is still quite limited, because most studies just assess a particular time frame in the invasion (Maron and Vilà 2001, Levine et al. 2004, Mitchell et al. 2006, Alofs and Jackson 2014, Papacostas et al. 2017). To our knowledge, our study is the first that reports the importance of exposure time for the development of resistance against invaders in natural ecosystems (but see Diez et al. 2010, Dostál et al. 2013 for assessments in common garden experiments) and highlights that increases in biotic resistance observed over time (because of increased consumer pressure, competitive impacts, or a combination of the two), could explain why invaders that have previously been considered as hypersuccessful (e.g., *Myriophyllum spicatum*, *Elodea canadensis*, *Dreissena polymorpha*, *Carcinus maenas*, *Caulerpa* species) can suffer marked reductions in population size some years after the onset of the invasion (Simberloff and Gibbons 2004, De Rivera et al. 2005, Ivesa et al. 2006, Carlsson and Strayer 2009, Bernardeau-Esteller et al. 2020). Therefore, by focusing on only a small time frame or just the beginning of an invasion, we may be underestimating the true capacity of native assemblages to develop resistance to invaders (Strayer et al. 2006, 2017, Rius et al. 2014, Papacostas et al. 2017). For this reason, the use of long-term data, despite being scarce, should be prioritized whenever possible, to assess the true effect that biotic resistance mechanisms might have on the overall invasion process.

Generally, our findings highlight the importance of considering several factors and their interaction when assessing the strength of biotic resistance mechanisms against a particular invader, especially considering that herbivory and competition are universal processes that operate across ecosystems and that naturally interact with each other (Gurevitch et al. 2000, Meiners and Handel 2000, Hambäck and Beckerman 2003). In fact, it has been proposed that herbivory reinforces competition and in turn releases the chance for coexistence, favoring those species that are better competitors (Gurevitch et al. 2000). However, despite that, in invasion ecology, the interaction between these mechanisms has been rarely assessed (but see Suwa and Louda 2012, Li et al. 2014, Zhang et al. 2018) and most studies rely on the assessment of single biotic mechanisms (Maron and Vilà 2001, Levine et al. 2004, Vilà and Weiner 2004, Kimbro et al. 2013, Papacostas et al. 2017 and references therein). This can definitively underestimate the true role of biotic processes (e.g., competition and herbivory) against invasive species and may explain why our findings, reporting a strong effect of biotic

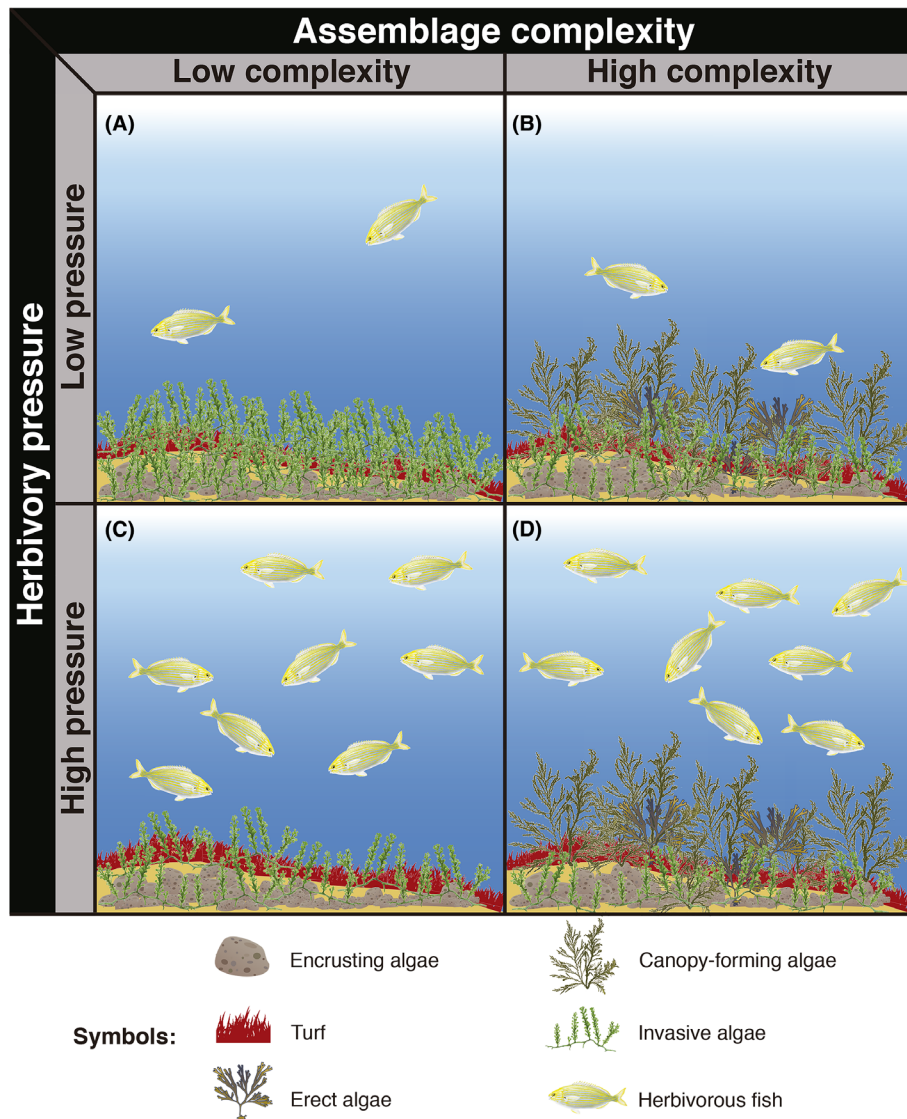


FIG. 5. Depiction of how different combinations of assemblage complexity and herbivory pressure determine biotic resistance against a marine invasive alga (e.g., *Caulerpa cylindracea*), representing four scenarios: (A) low assemblage complexity and low herbivory pressure; (B) high assemblage complexity and low herbivory pressure; (C) low assemblage complexity and high herbivory pressure and (D) high assemblage complexity and high herbivory pressure. (Algae illustrations were obtained and modified from the Integration & Application Network [IAN] Image Library [Tracey Saxby and Joanna Woerner], the IUCN, and freepik; <https://www.freepik.com/macrovectors>; the fish illustration is by João T. Tavares www.deviantart.com).

mechanisms against a successful invader, contrast with many previous studies. Actually, our results, together with those recently reported for mangrove ecosystems (Li et al. 2014, Zhang et al. 2018), where a successful invader (*Spartina alterniflora*) was limited and excluded due to the interaction between competition and herbivory, suggest that synergisms between biotic resistance mechanisms may be an important but overlooked process driving the invasion success of plant invaders (Fig. 6). In this sense, in situ field experiments, in which competition (e.g., assemblage complexity), herbivory, and

the interaction between the two can be simultaneously assessed, can provide a better understanding of the true extent of biotic resistance against an invader (Levine et al. 2004, Mitchell et al. 2006, Kimbro et al. 2013, Li et al. 2014, Enge et al. 2017, Zhang et al. 2018, Petruzzella et al. 2020) and will definitively help in the understanding of the invasion success and the dynamics of different invaders.

Finally, the findings reported in this study highlight the importance of improving our knowledge regarding the factors that influence invasive species success in

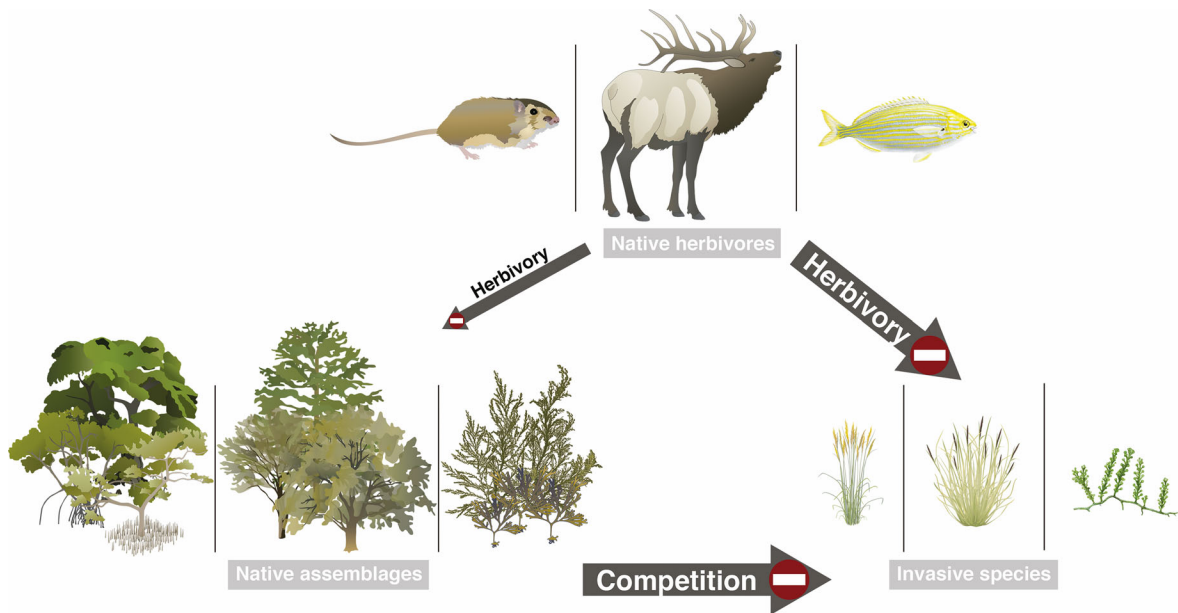


FIG. 6. Schematic representation of how the interaction between competition and herbivory might determine the overall biotic resistance against an invader. Depicted illustrations correspond to *Ratus losea*, *Cervus elaphus*, and *Sarpa salpa* as native herbivores; a mangrove forest, a temperate forest, and a marine algal forest as native assemblages; and two grasses and an alga species (here *Caulerpa cylindracea*) as invasive species. (Illustrations obtained from the Integration & Application Network [IAN] Image Library [Tracey Saxby, Kim Kraeer, and Lucy Van Essen-Fishman] and the IUCN, and the fish illustration is by João T. Tavares www.deviantart.com).

order to understand the invasion process of different species fully and adopt successful mitigation and management measures. As a practical example, although the removal of invasive algae has proven to be, in most cases, ineffective or infeasible (Epstein and Smale 2017, Giakoumi et al. 2019), results obtained in this and other studies (Bernardeau-Esteller et al. 2020) suggest that by promoting the conservation of marine habitats and herbivorous populations, we can foster biotic resistance within an ecosystem-based approach to marine environment management and contribute to the long-term control of marine invasions.

ACKNOWLEDGMENTS

The authors would like to thank Paula Molina, Cayetana Casas, Laura Royo, Eduardo Infantes, and Jana Verdura for help with field and laboratory work. Funding for this project was obtained from the Spanish Ministry Project ANIMA (CGL2016-76341-R, MINECO/FEDER, UE), the European Union's EMFS Program, AFRIMED (EASME/EMFF/2017/1.2.1.12/S4/01/SI2.789059), the Spanish Autonomous Organism of National Parks through the project BIGPARK (project 2017-2462), and the Padi Foundation to FT (CGA App: 5134). JS received the support of a fellowship from "La Caixa" Foundation (ID 100010434) with code (LCF/BQ/DE17/11600001). We thank the managers and rangers from the Cabrera Archipelago National Park for sampling permissions and helping with field logistics. This is a contribution from the Consolidated Research Group "Medrecover," SGR2017-1521 (Govern de Catalunya). Finally, we would like to thank M. Graham and the two anonymous reviewers for their valuable comments that

greatly improved the manuscript. Authors' contributions: FT and EC conceived the ideas and the experimental design; JS, FT, EB, JMR, JT, and EC carried out the fieldwork experiments and collected the data; JS analyzed the data; JS and EC drafted the manuscript, and all the authors contributed substantially to revisions and accepted the final version before submission.

LITERATURE CITED

- Alofs, K. M., and D. A. Jackson. 2014. Meta-analysis suggests biotic resistance in freshwater environments is driven by consumption rather than competition. *Ecology* 95:3259–3270.
- Amsberry, L., M. A. Baker, P. J. Ewanchuk, and M. D. Bertness. 2000. Clonal integration and the expansion of *Phragmites australis*. *Ecological Applications* 10:1110–1118.
- Anton, A., et al. 2019. Global ecological impacts of marine exotic species. *Nature Ecology and Evolution* 3:787–800.
- Arenas, F., I. Sánchez, S. J. Hawkins, and S. R. Jenkins. 2006. The invasibility of marine algal assemblages: Role of functional diversity and identity. *Ecology* 87:2851–2861.
- Ballesteros, E. 1986. Métodos de análisis estructural en comunidades naturales, en particular del fitobentos. *Oecologia Aquatica* 8:117–131.
- Ballesteros, E., M. Zabala, A. García-Rubies, and X. Turon. 1993. El bentos: Les comunitats. Pages 687–730 in J. A. Alcover, E. Ballesteros, and J. J. Fornós, editors. *Història natural de l'Arxipèlag de Cabrera*. Editorial Moll-CSIC, Palma de Mallorca, Spain.
- Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67:1–48.
- Bellard, C., P. Cassey, and T. M. Blackburn. 2016. Alien species as a driver of recent extinctions. *Biology Letters* 12:20150623.

- Bernardeau-Esteller, J., L. Marín-Guirao, J. M. Sandoval-Gil, R. García-Muñoz, A. Ramos-Segura, and J. Manuel Ruiz. 2020. Evidence for the long-term resistance of *Posidonia oceanica* meadows to *Caulerpa cylindracea* invasion. *Aquatic Botany* 160:103167.
- Bernardeau-Esteller, J., J. M. Ruiz, F. Tomas, J. M. Sandoval-Gil, and L. Marín-Guirao. 2015. Photoacclimation of *Caulerpa cylindracea*: Light as a limiting factor in the invasion of native Mediterranean seagrass meadows. *Journal of Experimental Marine Biology and Ecology* 465:130–141.
- Boudouresque, C. F. 1971. Méthodes d'étude qualitative et quantitative du benthos (en particulier du phytobenthos). *Tethys* 3:79–104.
- Box, A., S. Deudero, A. Sureda, A. Blanco, J. Alòs, J. Terrados, A. M. Grau, and F. Riera. 2009. Diet and physiological responses of *Spondyliosoma cantharus* (Linnaeus, 1758) to the *Caulerpa racemosa* var. *cylindracea* invasion. *Journal of Experimental Marine Biology and Ecology* 380:11–19.
- Britton-Simmons, K. H. 2006. Functional group diversity, resource preemption and the genesis of invasion resistance in a community of marine algae. *Oikos* 113:395–401.
- Bulleri, F., D. Balata, I. Bertocci, L. Tamburello, and L. Benedetti-Cecchi. 2010. The seaweed *Caulerpa racemosa* on Mediterranean rocky reefs: From passenger to driver of ecological change. *Ecology* 91:2205–2212.
- Bulleri, F., and L. Benedetti-Cecchi. 2008. Facilitation of the introduced green alga *Caulerpa racemosa* by resident algal turfs: Experimental evaluation of underlying mechanisms. *Marine Ecology Progress Series* 364:77–86.
- Bulleri, F., L. Tamburello, and L. Benedetti-Cecchi. 2009. Loss of consumers alters the effects of resident assemblages on the local spread of an introduced macroalga. *Oikos* 118:269–279.
- Byun, C., S. de Blois, and J. Brisson. 2013. Plant functional group identity and diversity determine biotic resistance to invasion by an exotic grass. *Journal of Ecology* 101:128–139.
- Byun, C., S. de Blois, and J. Brisson. 2015. Interactions between abiotic constraint, propagule pressure, and biotic resistance regulate plant invasion. *Oecologia* 178:285–296.
- Carlsson, N. O. L., O. Sarnelle, and D. L. Strayer. 2009. Native predators and exotic prey—An acquired taste? *Frontiers in Ecology and the Environment* 7:525–532.
- Carlsson, N. O. L., and D. L. Strayer. 2009. Intraspecific variation in the consumption of exotic prey—a mechanism that increases biotic resistance against invasive species? *Freshwater Biology* 54:2315–2319.
- Cebrian, E., and E. Ballesteros. 2009. Temporal and spatial variability in shallow- and deep-water populations of the invasive *Caulerpa racemosa* var. *cylindracea* in the Western Mediterranean. *Estuarine, Coastal and Shelf Science* 83:469–474.
- Cebrian, E., E. Ballesteros, C. Linares, and F. Tomas. 2011. Do native herbivores provide resistance to Mediterranean marine bioinvasions? A seaweed example. *Biological Invasions* 13:1397–1408.
- Ceccherelli, G., L. Piazza, and D. Balata. 2002. Spread of introduced *Caulerpa* species in macroalgal habitats. *Journal of Experimental Marine Biology and Ecology* 280:1–11.
- Coll, J. 2020. Seguiment de les comunitats marines d'Espais Naturals Protegits de les Illes Balears. Cens de poblacions de peixos (PN Cabrera). Informe tècnic per a la Direcció General d'Espais Naturals i Biodiversitat del Govern de les Illes Balears.
- Coll, M., et al. 2010. The biodiversity of the Mediterranean Sea: Estimates, patterns, and threats. *PLoS One* 5:e11842.
- Corbin, J. D., and C. M. D'Antonio. 2004. Competition between native perennial and exotic annual grasses: Implications for an historical invasion. *Ecology* 85:1273–1283.
- Cushman, J. H., C. J. Lortie, and C. E. Christian. 2011. Native herbivores and plant facilitation mediate the performance and distribution of an invasive exotic grass. *Journal of Ecology* 99:524–531.
- De Rivera, C. E., G. M. Ruiz, A. H. Hines, and P. Jivoff. 2005. Biotic resistance to invasion: Native predator limits abundance and distribution of an introduced crab. *Ecology* 86:3364–3376.
- Diez, J. M., I. Dickie, G. Edwards, P. E. Hulme, J. J. Sullivan, and R. P. Duncan. 2010. Negative soil feedbacks accumulate over time for non-native plant species. *Ecology Letters* 13:803–809.
- Dostál, P., J. Müllerová, P. Pyšek, J. Pergl, and T. Klinerová. 2013. The impact of an invasive plant changes over time. *Ecology Letters* 16:1277–1284.
- Elton, C. S. 1958. The ecology of invasions by animals and plants. University of Chicago Press, Chicago, Illinois, USA.
- Ender, C. L., C. E. Christian, and J. H. Cushman. 2017. Native herbivores and environmental heterogeneity as mediators of an exotic grass invasion. *Ecology and Evolution* 7:1561–1571.
- Enge, S., J. Sagerman, S. A. Wikström, and H. Pavia. 2017. A review of herbivore effects on seaweed invasions. *Oceanography and Marine Biology: An Annual Review* 55:421–440.
- Epstein, G., and D. A. Smale. 2017. *Undaria pinnatifida*: a case study to highlight challenges in marine invasion ecology and management. *Ecology and Evolution* 7:8624–8642.
- Eschtruth, A. K., and J. J. Battles. 2009a. Assessing the relative importance of disturbance, herbivory, diversity, and propagule pressure in exotic plant invasion. *Ecological Monographs* 79:265–280.
- Eschtruth, A. K., and J. J. Battles. 2009b. Acceleration of exotic plant invasion in a forested ecosystem by a generalist herbivore. *Conservation Biology* 23:388–399.
- Fine, P. V. A. 2002. The invasibility of tropical forests by exotic plants. *Journal of Tropical Ecology* 18:687–705.
- Forslund, H., S. A. Wikström, and H. Pavia. 2010. Higher resistance to herbivory in introduced compared to native populations of a seaweed. *Oecologia* 164:833–840.
- Galil, B. S. 2007. Loss or gain? Invasive aliens and biodiversity in the Mediterranean Sea. *Marine Pollution Bulletin* 55:314–322.
- Giakoumi, S., et al. 2019. Management priorities for marine invasive species. *Science of the Total Environment* 688:976–982.
- Gianni, F., F. Bartolini, A. Pey, M. Laurent, G. M. Martins, L. Airolidi, and L. Mangialajo. 2017. Threats to large brown algal forests in temperate seas: the overlooked role of native herbivorous fish. *Scientific Reports* 7:1–13.
- Grutters, B. M. C., Y. O. A. Roijendijk, W. C. E. P. Verberk, and E. S. Bakker. 2017. Plant traits and plant biogeography control the biotic resistance provided by generalist herbivores. *Functional Ecology* 31:1184–1192.
- Guidetti, P., et al. 2014. Large-scale assessment of Mediterranean marine protected areas effects on fish assemblages. *PLoS One* 9:e91841.
- Gurevitch, J., J. A. Morrison, and L. V. Hedges. 2000. The interaction between competition and predation: A meta-analysis of field experiments. *American Naturalist* 155:435–453.
- Hambäck, P. A., and A. P. Beckerman. 2003. Herbivory and plant resource competition: A review of two interacting interactions. *Oikos* 101:26–37.
- Havelange, S., G. Lepoint, P. Dauby, and J. M. Bouqueneau. 1997. Feeding of the sparid fish *Sarpa salpa* in a seagrass ecosystem: diet and carbon flux. *Marine Ecology* 18:289–297.
- Inderjit, M. R. Chapman, and S. Kaushik. 2006. Invasive marine algae: An ecological perspective. *Botanical Review* 72:26.

- Iveša, L., A. Jaklin, and M. Devescovi. 2006. Vegetation patterns and spontaneous regression of *Caulerpa taxifolia* (Vahl) C. Agardh in Malinska (Northern Adriatic, Croatia). *Aquatic Botany* 85:324–330.
- Kalisz, S., R. B. Spigler, and C. C. Horvitz. 2014. In a long-term experimental demography study, excluding ungulates reversed invader's explosive population growth rate and restored natives. *Proceedings of the National Academy of Sciences of the United States of America* 111:4501–4506.
- Katsanevakis, S., F. Tempera, and H. Teixeira. 2016. Mapping the impact of alien species on marine ecosystems: The Mediterranean Sea case study. *Diversity and Distributions* 22:694–707.
- Keane, R. M., and M. J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution* 17:164–170.
- Kimbrow, D. L., B. S. Cheng, and E. D. Grosholz. 2013. Biotic resistance in marine environments. *Ecology Letters* 16:821–833.
- Klein, J., and M. Verlaque. 2008. The *Caulerpa racemosa* invasion: A critical review. *Marine Pollution Bulletin* 56:205–225.
- Lenth, R. 2018. emmeans: Estimated marginal means, aka least-squares means. R package version 1.3.0. <https://cran.r-project.org/web/packages/emmeans/index.html>
- Levine, J. M., P. B. Adler, and S. G. Yelenik. 2004. A meta-analysis of biotic resistance to exotic plant invasions. *Ecology Letters* 7:975–989.
- Li, Z., W. Wang, and Y. Zhang. 2014. Recruitment and herbivory affect spread of invasive *Spartina alterniflora* in China. *Ecology* 95:1972–1980.
- Lindig-Cisneros, R., and J. B. Zedler. 2002. Relationships between canopy complexity and germination microsites for *Phalaris arundinacea* L. *Oecologia* 133:159–167.
- Liu, H., and P. Stiling. 2006. Testing the enemy release hypothesis: a review and meta-analysis. *Biological Invasions* 8:1535–1545.
- Marco-Méndez, C., L. M. Ferrero-Vicente, P. Prado, and J. L. Sánchez-Lizaso. 2017. Epiphytes and nutrient contents influence *Sarpa salpa* herbivory on *Caulerpa* spp. vs. seagrass species in Mediterranean meadows. *Estuarine, Coastal and Shelf Science* 184:54–66.
- Marín-Guirao, L., J. Bernardeau-Esteller, J. M. Ruiz, and J. M. Sandoval-Gil. 2015. Resistance of *Posidonia oceanica* seagrass meadows to the spread of the introduced green alga *Caulerpa cylindracea*: assessment of the role of light. *Biological Invasions* 17:1989–2009.
- Maron, J. L., and M. Vilà. 2001. When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos* 95:361–373.
- Meiners, S. J., and S. N. Handel. 2000. Additive and nonadditive effects of herbivory and competition on tree seedling mortality, growth, and allocation. *American Journal of Botany* 87:1821–1826.
- Mitchell, C. E., et al. 2006. Biotic interactions and plant invasions. *Ecology Letters* 9:726–740.
- Morris, T. L., N. N. Barger, and M. D. Cramer. 2015. Competitive resistance of a native shrubland to invasion by the alien invasive tree species, *Acacia cyclops*. *Biological Invasions* 17:3563–3577.
- Nejrup, L. B., M. F. Pedersen, and J. Vinzent. 2012. Grazer avoidance may explain the invasiveness of the red alga *Gracilaria vermiculophylla* in Scandinavian waters. *Marine Biology* 159:1703–1712.
- Papacostas, K. J., E. W. Rielly-Carroll, S. E. Georgian, D. J. Long, S. D. Princiotta, A. M. Quattrini, K. E. Reuter, and A. L. Freestone. 2017. Biological mechanisms of marine invasions. *Marine Ecology Progress Series* 565:251–268.
- Parker, J. D., D. E. Burkepile, and M. E. Hay. 2006. Opposing effects of native and exotic herbivores on plant invasions. *Science* 311:1459–1461.
- Parker, J. D., and M. E. Hay. 2005. Biotic resistance to plant invasions? Native herbivores prefer non-native plants. *Ecology Letters* 8:959–967.
- Pearson, D. E., T. Potter, and J. L. Maron. 2012. Biotic resistance: Exclusion of native rodent consumers releases populations of a weak invader. *Journal of Ecology* 100:1383–1390.
- Pejchar, L., and H. A. Mooney. 2009. Invasive species, ecosystem services and human well-being. *Trends in Ecology and Evolution* 24:497–504.
- Perelman, S. B., E. J. Chaneton, W. B. Batista, S. E. Burkart, and R. J. C. León. 2007. Habitat stress, species pool size and biotic resistance influence exotic plant richness in the Flooding Pampa grasslands. *Journal of Ecology* 95:662–673.
- Petrizzella, A., J. Manschot, C. H. A. Van Leeuwen, B. M. C. Grutters, and E. S. Bakker. 2018. Mechanisms of invasion resistance of aquatic plant communities. *Frontiers in Plant Science* 9:134.
- Petrizzella, A., C. H. A. van Leeuwen, E. van Donk, and E. S. Bakker. 2020. Direct and indirect effects of native plants and herbivores on biotic resistance to alien aquatic plant invasions. *Journal of Ecology* 108:1487–1496.
- Piazzi, L., and D. Balata. 2009. Invasion of alien macroalgae in different Mediterranean habitats. *Biological Invasions* 11:193–204.
- Piazzi, L., D. Balata, F. Bulleri, P. Gennaro, and G. Ceccherelli. 2016. The invasion of *Caulerpa cylindracea* in the Mediterranean: The known, the unknown and the knowable. *Marine Biology* 163:161.
- Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* 52:273–288.
- Pokorny, M. L., R. L. Sheley, C. A. Zabinski, R. E. Engel, T. J. Svejcar, and J. J. Borkowski. 2005. Plant functional group diversity as a mechanism for invasion resistance. *Restoration Ecology* 13:448–459.
- Poore, A. G. B., et al. 2012. Global patterns in the impact of marine herbivores on benthic primary producers. *Ecology Letters* 15:912–922.
- R Development Core Team. 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. www.r-project.org
- Relva, M. A., M. A. Nuñez, and D. Simberloff. 2010. Introduced deer reduce native plant cover and facilitate invasion of non-native tree species: Evidence for invasional meltdown. *Biological Invasions* 12:303–311.
- Reñones, O., J. Moranta, J. Coll, and B. Morales-Nin. 1997. Rocky bottom fish communities of Cabrera Archipelago National Park (Mallorca, Western Mediterranean). *Scientia Marina* 61:495–506.
- Rius, M., E. E. Potter, J. D. Aguirre, and J. J. Stachowicz. 2014. Mechanisms of biotic resistance across complex life cycles. *Journal of Animal Ecology* 83:296–305.
- Ruitton, S., M. Verlaque, G. Aubin, and C. F. Boudouresque. 2006. Grazing on *Caulerpa racemosa* var. *cylindracea* (Caulerpaceae, Chlorophyta) in the Mediterranean Sea by herbivorous fishes and sea urchins. *Vie et Milieu* 56:33–41.
- Sala, E., et al. 2012. The structure of Mediterranean rocky reef ecosystems across environmental and human gradients, and conservation implications. *PLoS One* 7:e32742.
- Sala, E., and E. Ballesteros. 1997. Partitioning of space and food resources by three fish of the genus *Diplodus* (Sparidae) in a Mediterranean rocky infralittoral ecosystem. *Marine Ecology Progress Series* 152:273–283.

- Sant, N., E. Chappuis, C. Rodríguez-Prieto, M. Real, and E. Ballesteros. 2017. Cost-benefit of three different methods for studying Mediterranean rocky benthic assemblages. *Scientia Marina* 81:129.
- Santamaría, J. 2021. JorgeSantamaria/Biotic-Mechanisms-Algae-Invasions: First release of data and code to assess biotic mechanisms. (Version v1.0.0). Ecology. Zenodo. <https://doi.org/10.5281/zenodo.4664432>
- Schaffelke, B., J. E. Smith, and C. L. Hewitt. 2006. Introduced macroalgae—A growing concern. *Journal of Applied Phycology* 18:529–541.
- Simberloff, D., et al. 2013. Impacts of biological invasions: What's what and the way forward. *Trends in Ecology and Evolution* 28:58–66.
- Simberloff, D., and L. Gibbons. 2004. Now you see them, now you don't!—population crashes of established introduced species. *Biological Invasions* 6:161–172.
- Steneck, R. S., D. R. Bellwood, and M. E. Hay. 2017. Herbivory in the marine realm. *Current Biology* 27:R484–R489.
- Stohlgren, T. J., L. D. Schell, and B. Vanden Heuvel. 1999. How grazing and soil quality affect native and exotic plant diversity in rocky mountain grasslands. *Ecological Applications* 9:45–64.
- Strayer, D. L., et al. 2017. Boom–bust dynamics in biological invasions: Towards an improved application of the concept. *Ecology Letters* 20:1337–1350.
- Strayer, D. L., V. T. Eviner, J. M. Jeschke, and M. L. Pace. 2006. Understanding the long-term effects of species invasions. *Trends in Ecology and Evolution* 21:645–651.
- Suwa, T., and S. M. Louda. 2012. Combined effects of plant competition and insect herbivory hinder invasiveness of an introduced thistle. *Oecologia* 169:467–476.
- te Beest, M., N. J. Mpandza, and H. Olff. 2018. Biotic resistance affects growth and reproduction, but not survival of a high-impact woody invader in African savannas. *Journal of Vegetation Science* 29:532–540.
- Theoharides, K. A., and J. S. Dukes. 2007. Plant invasion across space and time: Factors affecting nonindigenous species success during four stages of invasion. *New Phytologist* 176:256–273.
- Thomsen, M. S., T. Wernberg, P. M. South, and D. R. Schiel. 2016. Non-native seaweeds drive changes in marine coastal communities around the world. Pages 147–185 in Z. M. Hu, and C. Fraser, editors. *Seaweed phylogeography: adaptation and evolution of seaweeds under environmental change*. Springer, Dordrecht, Netherlands.
- Tomas, F., A. Box, and J. Terrados. 2011a. Effects of invasive seaweeds on feeding preference and performance of a key-stone Mediterranean herbivore. *Biological Invasions* 13:1559–1570.
- Tomas, F., E. Cebrian, and E. Ballesteros. 2011b. Differential herbivory of invasive algae by native fish in the Mediterranean Sea. *Estuarine, Coastal and Shelf Science* 92:27–34.
- Trygonis, V., and M. Sini. 2012. photoQuad: a dedicated seabed image processing software, and a comparative error analysis of four photoquadrat methods. *Journal of Experimental Marine Biology and Ecology* 424–425:99–108.
- Vaz-Pinto, F., C. Olabarria, and F. Arenas. 2012. Propagule pressure and functional diversity: interactive effects on a macroalgal invasion process. *Marine Ecology Progress Series* 471:51–60.
- Vergés, A., T. Alcoverro, and E. Ballesteros. 2009. Role of fish herbivory in structuring the vertical distribution of canopy algae *Cystoseira* spp. in the Mediterranean Sea. *Marine Ecology Progress Series* 375:1–11.
- Vergés, A., F. Tomas, and E. Ballesteros. 2012. Interactive effects of depth and marine protection on predation and herbivory patterns. *Marine Ecology Progress Series* 450:55–65.
- Verlaque, M. 1990. Relations entre *Sarpa salpa* (Linnaeus, 1758) (Téléostéen, Sparidae), les autres poissons broyeurs et le phytobenthos algal méditerranéen. *Oceanologica Acta* 13:373–388.
- Verlaque, M., J. Afonso-Carrillo, M. C. Gil-Rodríguez, C. Durand, C. F. Boudouresque, and Y. Le Parco. 2004. Blitzkrieg in a marine invasion: *Caulerpa racemosa* var. *cylindracea* (Bryopsidales, Chlorophyta) reaches the Canary Islands (North-East Atlantic). *Biological Invasions* 6:269–281.
- Verlaque, M., C. Durand, J. M. Huisman, C. F. Boudouresque, and Y. Le Parco. 2003. On the identity and origin of the Mediterranean invasive *Caulerpa racemosa* (Caulerpales, Chlorophyta). *European Journal of Phycology* 38:325–339.
- Vilà, M., et al. 2010. How well do we understand the impacts of alien species on ecosystem services? A pan-European, cross-taxa assessment. *Frontiers in Ecology and the Environment* 8:135–144.
- Vilà, M., J. L. Espinar, M. Hejda, P. E. Hulme, V. Jarosik, J. L. Maron, J. Pergl, U. Schaffner, Y. Sun, and P. Pyšek. 2011. Ecological impacts of invasive alien plants: A meta-analysis of their effects on species, communities and ecosystems. *Ecology Letters* 14:702–708.
- Vilà, M., and J. Weiner. 2004. Are invasive plant species better competitors than native plant species?—Evidence from pairwise experiments. *Oikos* 105:229–238.
- Wikström, S. A., M. B. Steinarsdóttir, L. Kautsky, and H. Pavia. 2006. Increased chemical resistance explains low herbivore colonization of introduced seaweed. *Oecologia* 148:593–601.
- Williams, S. L., and J. E. Smith. 2007. A global review of the distribution, taxonomy, and impacts of introduced seaweeds. *Annual Review of Ecology, Evolution and Systematics* 38:327–359.
- Zhang, Y., H. Meng, Y. Wang, and Q. He. 2018. Herbivory enhances the resistance of mangrove forest to cordgrass invasion. *Ecology* 99:1382–1390.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at <http://onlinelibrary.wiley.com/doi/10.1002/ecy.3440/supinfo>

OPEN RESEARCH

R code and data (Santamaría 2021) for this publication are available on Zenodo: <https://doi.org/10.5281/zenodo.4664432>.